

Exhibit A

Clean Version of The Pending Claims in U.S. Patent Application Ser. No. 09/870,113

1. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, and 12.

3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2.

4. (New) The isolated nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1.

5. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:4.

6. (New) The isolated nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3.

7. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:6.

8. (New) The isolated nucleic acid molecule of claim 7, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:5.

9. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:12.

10. (New) The isolated nucleic acid molecule of claim 9, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:11.

11. (New) A recombinant expression vector comprising the isolated nucleic acid molecule of claim 1.

12. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1.

13. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3.

14. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:5.

15. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:11.

16. (New) A host cell comprising the recombinant expression vector of claim 11.

Exhibit B

Marked Up Version of Amended Claims in U.S. Patent Application Ser. No. 09/870,113

1. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence encoding an amino acid sequence [drawn] selected from the group consisting of SEQ ID NOS:2, 4, 6, [8, 10,] and 12.
2. (Cancelled) An isolated nucleic acid molecule comprising a nucleotide sequence that:
 - (a) encodes the amino acid sequence shown in SEQ ID NO:2; and
 - (b) hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:1 or the complement thereof.
3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2.
4. (New) The isolated nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1.
5. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:4.
6. (New) The isolated nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3.
7. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:6.
8. (New) The isolated nucleic acid molecule of claim 7, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:5.

9. (New) The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:12.

10. (New) The isolated nucleic acid molecule of claim 9, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:11.

11. (New) A recombinant expression vector comprising the isolated nucleic acid molecule of claim 1.

12. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1.

13. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3.

14. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:5.

15. (New) The recombinant expression vector of claim 11, wherein said nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:11.

16. (New) A host cell comprising the recombinant expression vector of claim 11.

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variant
Length = 1448

Score = 760 bits (1940), Expect = 0.0
Identities = 364/364 (100%), Positives = 364/364 (100%)
Frame = +3

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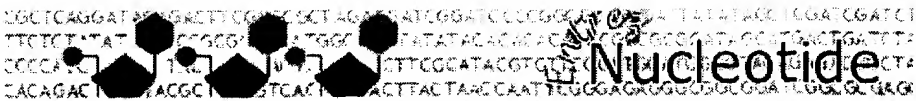
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[Genome](#)
[Structure](#)
[PMC](#)
[Taxonomy](#)
[OMIM](#)
[Boo](#)

Search for

1: AJ303077. Homo sapiens mRNA...[gi:12666719]

[Links](#)

LOCUS HSA303077 1448 bp mRNA linear PRI 26-APR-2001
DEFINITION Homo sapiens mRNA for mitochondrial RNA splicing protein 3/4 (HMRS3/4 gene), 1448 bp cDNA splice variant.
ACCESSION AJ303077
VERSION AJ303077.1 GI:12666719
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 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1
AUTHORS Li,F.Y., Nikali,K., Gregan,J., Leibiger,I., Leibiger,B., Schweyen,R., Larsson,C. and Suomalainen,A.
TITLE Characterization of a novel human putative mitochondrial transporter homologous to the yeast mitochondrial RNA splicing proteins 3 and 4
JOURNAL FEBS Lett. 494 (1-2), 79-84 (2001)
MEDLINE 21195335
PUBMED 11297739
REFERENCE 2 (bases 1 to 1448)
AUTHORS Nikali,K.
TITLE Direct Submission
JOURNAL Submitted (12-DEC-2000) Nikali K., Human Molecular Genetics, National Public Health Institute, Mannerheimintie 166, Helsinki, FI-00300, FINLAND
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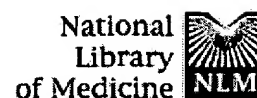
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May 2 2003 16:47:12



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Genome

Structure

PMC

Taxonomy

OMIM

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☐ 1: FEBS Lett 2001 Apr 6;494(1-2):79-84

Related Articles, Link

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Characterization of a novel human putative mitochondrial transporter homologous to the yeast mitochondrial RNA splicing proteins 3 and 4.

Li FY, Nikali K, Gregan J, Leibiger I, Leibiger B, Schweyen R, Larsson C, Suomalainen A.

Department of Molecular Medicine, CMM, Karolinska Hospital, Stockholm, Sweden. fangyuan.li@cmm.ki.se

We report here a novel human gene, hMRS3/4, encoding a putative mitochondrial transporter structurally and functionally homologous to the yeast mitochondrial RNA splicing proteins 3 and 4. These proteins belong to the family of mitochondrial carrier proteins (MCF) and are likely to function as solute carriers. hMRS3/4 spans approximately 10 kb of genomic DNA on chromosome 10q24 and consists of four exons that encode a 364-aa protein with six transmembrane domains. A putative splice variant, encoding a 177-aa protein with three transmembrane domains, was also identified. hMRS3/4 has a well-conserved signature sequence of MCF and is targeted into the mitochondria. When expressed in yeast, hMRS3/4 efficiently restores the mitochondrial functions in mrs3(o)mrs4(o) knock-out mutants. Ubiquitous expression in human tissues and a well-conserved structure and function suggest an important role for hMRS3/4 in human cells.

MeSH Terms:

- Alternative Splicing*
- Amino Acid Sequence
- Base Sequence
- Carrier Proteins/metabolism
- Carrier Proteins/genetics*
- Chromosome Mapping
- Chromosomes, Human, Pair 10*
- DNA, Complementary
- Fungal Proteins/genetics
- Gene Expression Profiling
- Human
- Mitochondria/metabolism*

- Molecular Sequence Data
- Mutagenesis
- Ophthalmoplegia, Chronic Progressive External/genetics
- RNA Splicing
- Saccharomyces cerevisiae/genetics
- Spinocerebellar Ataxias/genetics
- Support, Non-U.S. Gov't
- Tissue Distribution
- Transfection

Substances:

- MRS3 protein, S cerevisiae
- MRS4 protein, S cerevisiae
- HMRS3-4 protein, human
- Fungal Proteins
- DNA, Complementary
- Carrier Proteins

Secondary source id:

- GENBANK/AJ303078
- GENBANK/AJ303077
- GENBANK/AF327403
- GENBANK/AF327402

PMID: 11297739 [PubMed - indexed for MEDLINE]

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May 2 2003 16:34:2

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PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

OMIM

Bio

 Search for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

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Send to

Get Subsequence

☐ 1: AL353719. Human DNA sequenc...[gi:15787725]

Links

LOCUS AL353719 123160 bp DNA linear PRI 25-SEP-2001
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 REFERENCE 1 (bases 1 to 123160)
 AUTHORS Ramsay, H.
 TITLE Direct Submission
 JOURNAL Submitted (25-SEP-2001) Sanger Centre, Hinxton, Cambridgeshire, CB10 1SA, UK. E-mail enquiries: humquery@sanger.ac.uk Clone requests: clonerequest@sanger.ac.uk
 COMMENT On Sep 26, 2001 this sequence version replaced gi:14280413. During sequence assembly data is compared from overlapping clones. Where differences are found these are annotated as variations together with a note of the overlapping clone name. Note that the variation annotation may not be found in the sequence submission corresponding to the overlapping clone, as we submit sequences with only a small overlap as described above.
 This sequence was finished as follows unless otherwise noted: all regions were either double-stranded or sequenced with an alternate chemistry or covered by high quality data (i.e., phred quality >= 30); an attempt was made to resolve all sequencing problems, such as compressions and repeats; all regions were covered by at least one plasmid subclone or more than one M13 subclone; and the assembly was confirmed by restriction digest. The following abbreviations are used to associate primary accession numbers given in the feature table with their source databases: Em:, EMBL; Sw:, SWISSPROT; Tr:, TREMBL; Wp:, WORMPEP; Information on the WORMPEP database can be found at http://www.sanger.ac.uk/Projects/C_elegans/wormpep This sequence was generated from part of bacterial clone contigs of human chromosome 10, constructed by the Sanger Centre Chromosome 10 Mapping Group. Further information can be found at <http://www.sanger.ac.uk/HGP/Chr10> RP11-85A1 is from the library RPCI-11.1 constructed by the group of Pieter de Jong. For further details see <http://www.chori.org/bacpac/home.htm>
 VECTOR: pBACe3.6
 IMPORTANT: This sequence is not the entire insert of clone RP11-85A1 It may be shorter because we sequence overlapping sections only once, except for a short overlap. The true left end of clone RP11-85A1 is at 1 in this sequence. The

Characterization of a novel human putative mitochondrial transporter homologous to the yeast mitochondrial RNA splicing proteins 3 and 4

Fang-Yuan Li^{a,1,*}, Kaisu Nikali^{b,1}, Juraj Gregan^c, Ingo Leibiger^d, Barbara Leibiger^d, Rudolf Schweyen^c, Catharina Larsson^a, Anu Suomalainen^{b,e}

^aDepartment of Molecular Medicine, CMM, L8:01, Karolinska Hospital, 171 76 Stockholm, Sweden

^bDepartment of Human Molecular Genetics, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland

^cVienna Biocenter, Institute of Microbiology and Genetics, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria

^dDepartment of Molecular Medicine, The Rolf Luft Center for Diabetes Research L3, Karolinska Hospital, 171 76 Stockholm, Sweden

^eMontreal Neurological Institute, McGill University, 3801 rue University, Montreal, QC, Canada H3A 2B4

Received 20 December 2000; revised 9 March 2001; accepted 12 March 2001

First published online 23 March 2001

Edited by Matti Saraste

Abstract We report here a novel human gene, *hMRS3/4*, encoding a putative mitochondrial transporter structurally and functionally homologous to the yeast mitochondrial RNA splicing proteins 3 and 4. These proteins belong to the family of mitochondrial carrier proteins (MCF) and are likely to function as solute carriers. *hMRS3/4* spans ~10 kb of genomic DNA on chromosome 10q24 and consists of four exons that encode a 364-aa protein with six transmembrane domains. A putative splice variant, encoding a 177-aa protein with three transmembrane domains, was also identified. *hMRS3/4* has a well-conserved signature sequence of MCF and is targeted into the mitochondria. When expressed in yeast, *hMRS3/4* efficiently restores the mitochondrial functions in *mrs3⁰mrs4⁰* knock-out mutants. Ubiquitous expression in human tissues and a well-conserved structure and function suggest an important role for *hMRS3/4* in human cells. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Mitochondrial carrier; Solute transport; MRS3; MRS4; Chromosome 10

1. Introduction

The mitochondrial proteins MRS3 and MRS4 (yMRS3 and yMRS4) were first identified in *Saccharomyces cerevisiae* in 1991 by their ability to suppress defects in the maturation of mitochondrial group II intron-containing transcripts [1]. Subsequently, they were found to be integral membrane proteins in the inner mitochondrial membrane, belonging to the superfamily of mitochondrial carriers (MCF), and having structural features of solute carriers [2]. This superfamily includes the adenine nucleotide translocator, the phosphate carrier, several mitochondrial metabolite carriers, as well as other proteins of shared structure and unknown function ([3]; Protein family database, Pfam; Prosite accession number PDOC00189).

We have undertaken positional cloning projects to characterize the genes underlying autosomal dominant progressive

external ophthalmoplegia with multiple mtDNA deletions (adPEO) and infantile-onset spinocerebellar ataxia (IOSCA). The genes of one form of adPEO and IOSCA have been mapped to chromosome 10q24 between DNA markers D10S198 and D10S1795 [4,5], and D10S192 and D10S1265 [6,7], respectively. Expressed sequence tags (ESTs) weakly homologous to the yMRS3 and yMRS4 had previously been mapped in the immediate vicinity of this region.

Here we present the cloning and characterization of a novel human gene, *hMRS3/4*, encoding a putative mitochondrial transporter with significant structural homology to the closely related yMRS3 and yMRS4, and with ability to replace its homologs in yeast. Sequence analyses were carried out to clarify the possible involvement of *hMRS3/4* in adPEO and IOSCA diseases, sharing the genomic locus with this transporter.

2. Materials and methods

2.1. Construction of the *hMRS3/4* cDNA sequence

To construct the *hMRS3/4* cDNA sequence, 104 ESTs homologous with yMRS3 and yMRS4, and some of them previously localized to 10q24, were identified from GenBank. The putative *hMRS3/4* cDNA was constructed by aligning the overlapping ESTs (GenBank accession numbers AA234450, AA361317, AA234031, AA449277, AA298599 and AA298105) and cDNA sequences of corresponding IMAGE cDNA clone inserts (clone numbers 77169, 346560, and 1192817; 3' cDNA representing exons 2–4). In addition, cDNA sequence information was gained by exon predictions and homology comparisons between yMRS4 and the available genomic sequence of *hMRS3/4* locus (GenBank accession number AC007643). The ESTs represented two different splice forms, and the cDNAs constructed from these had 177-aa and 364-aa open reading frames (ORF). The two forms shared the 3' UTR and exon 4, but had differential splicing of exons 2 and 3 (Fig. 1). About 13% of the ESTs corresponded to the 177-aa form. The rest were either homologous with the 364-aa form or too short to extend to the variable sequence. The constructed *hMRS3/4* cDNAs and the correct genomic sequence were confirmed by polymerase chain reaction (PCR) amplification of reverse-transcribed muscle, brain or lymphoblast RNA, of genomic DNA, and by subsequent sequencing. Table 1 shows the PCR primers used. PCR amplifications were carried out with either Dynazyme II DNA polymerase (Finnzymes, Finland) or the High Fidelity PCR Master kit (Boehringer-Mannheim-Roche) according to the instructions of the manufacturers and with primer-specific annealing temperatures. BigDye Terminator cycle sequencing protocol (Perkin Elmer) was used for sequencing reactions, and subsequent sequence analyses of both DNA strands were carried out on ABI377 sequencer (Perkin Elmer).

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2.2. Genomic structure of the *hMRS3/4* gene

The exact genomic structure of *hMRS3/4* was determined by comparison of the cDNA sequence to the genomic sequence obtained by DNA sequencing or by searching public databases. Recently, the entire genomic sequence of the *hMRS3/4* locus became available in GenBank (accession number AC007643).

2.3. Computer analyses

All sequences were aligned using Sequencher 3.0 software (Gene Codes Corporation, Ann Arbor, MI, USA) or GeneJockey II (Bio-soft, Cambridge, UK). Nucleotide and protein sequence searches were carried out with BLAST algorithms against public databases. Exon predictions from genomic sequence were carried out using programs EbEST, Genexpress, GeneJockey II, and Genscan. Programs ORF Finder and eukaryotic ORF ID at BCM Search Launcher were used in predicting ORFs from the cDNA sequence. Programs Predict-Protein, ProfileScan, ProDom, PSORT II, ScanProsite, and TMpred were used in predicting the potential features of *hMRS3/4*. (<http://web.wi.mit.edu/bio/pub/biopage2.html>).

2.4. Refined chromosomal assignment of *hMRS3/4* gene

The presence of *hMRS3/4* sequence in a panel of 83 radiation hybrids (RH, Radiation Hybrid Panel G3, RH01.05, Research Genetics, Huntsville, AL, USA) and in a YAC contig on the 10q24, constructed from the publicly available mega-YACs (CEPH), was analyzed by PCR amplification using primers MRS4-5+MRS4-8 and 7875F+8284R (Table 1). The PCR products were visualized by 1% agarose gel electrophoresis.

2.5. Sequence analyses in patient and control subjects

The 3.2-kb genomic sequence of the 3'-end of *hMRS3/4* was amplified in three overlapping fragments (primer pairs 8284R+10636F, 10634R+1805F2, and 10637R+1805F2; Table 1), and the first exon in a single fragment (primers MRS4ex1F+R; Table 1), from the DNA samples of two adPEO patients, originating from both a Finnish and a Pakistani adPEO family both linked to chromosome 10q24 [4,5], one IOSCA patient from a family also linked to 10q24 [6,7], and two control subjects. Both DNA strands of the PCR fragments were sequenced with PCR and internal primers by ABI377 BigDye Terminator cycle sequencing protocol according to manufacturer's instructions. To enhance detection of a heterozygous mutation, most of the PCR fragments were additionally analyzed by single-strand conformation polymorphism (SSCP) analysis as previously described [8].

2.6. Northern blot analysis

To analyze the expression pattern of *hMRS3/4*, the 1.1-kb insert of IMAGE clone 1192817, PCR-amplified with vector primers and purified with Qiaquick PCR preps (Qiagen, Valencia, CA, USA) according to the instructions of the manufacturer, and the 1.1-kb RT-PCR product amplified with primers MRS4-20+21 from lymphoblast RNA template, were radioactively labeled by standard random-prime methodology (Fig. 1). Additionally, to detect specifically the 364-aa splice form, a 347-bp RT-PCR product amplified with MRS4-34+39 was labeled by integrating ³²P during PCR. These probes were hybridized separately to human multiple tissue Northern (MTN[®]) blot containing mRNAs from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 1192817 insert was also hybridized to human brain MTN-blot II containing mRNAs from eight different regions of the brain (Clontech, Palo Alto, CA, USA). Northern hybridizations were carried out in ExpressHyb solution (Clontech, Palo Alto, CA, USA) in accordance with the instructions of the manufacturer. After hybridizations, the membranes were exposed to X-ray film at -70°C overnight or for several days.

2.7. Mitochondrial targeting of *hMRS3/4*

The *hMRS3/4* cDNA encoding the 364-aa splice form, lacking only the non-conserved 5'-end, and the cDNA encoding the 177-aa variant, were amplified by high-fidelity PCR with primers MRS4-41+21 and MRS4-20 and -21, respectively (Table 1), and cloned into pCR2.1 (Invitrogen, San Diego, CA, USA). They were reamplified with MRS4-207 and -201, MRS4-200 and MRS4-201 to introduce an *EcoRV* and a *HindIII* site to facilitate cloning and to eliminate the stop codon. The product was subcloned into pB.RSV.PDX-1~GFP [9], fusing the *hMRS3/4* and EGFP (enhanced green fluorescence protein) cDNAs in-frame and creating pRSV.hMRS3/4-177~EGFP

Table 1

5'→3' sequences of primers used in PCR and sequencing

Name	Sequence
7875F	AGCCATCGCATGGTCTGTGT
8284R	GGAGAGAGGTTATCAAGGTGCTG
10634R	TGAGCAGTGTTTGCACACGTCC
10636F	CTCCACTGGTGGCAGGTGATGT
10637R	GGTTCATGGCTGCATCATGAAG
1805F2	CCTGACCCAGCTGCCCGCTAT
MRS4ex1F	CGCGCCTGAGCGGACACTA
MRS4ex1R	GGTAGTGGCAGGAGCCAGGAA
MRS4-5	GGGGTGCAGGCCAGAGTAAT
MRS4-8	AGGAAAGGTGGTGGCAACAG
MRS4-20	CTTGAGCTGGCCTTCCTATC
MRS4-21	AGGGGAATGAGCTGCTTATC
MRS4-34	CCCCTTCATTTTGCCACACT
MRS4-39	GCCCGCTATCGCAATGTGTT
MRS4-41	GCCCCCGGTACGACAAGATC
MRS4-200	CCGCATGATATCACCATGAACCTG
MRS4-201	GCTACTAAGCTTGCCAGCCCTCCAC
MRS4-207:	GTCACCGATATCATGTGGCAGGC
<i>hMRS4-SacI</i>	CACACGAGCTCATGCCCCGGTACGAC
<i>hMRS4-HindIII</i> :	CACACAAGCTTTCACCTGCCAGCCCTC

and pRSV.hMRS3/4-364~EGFP. The constructs were confirmed by DNA sequence analysis. HEK293 cells were grown on 24-mm glass coverslips in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/l glucose and supplemented (DMEM+) with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum at 5% CO₂ and 37°C. Transfections of pRSVhMRS3/4-177 and -364~EGFP by lipofectamine were performed overnight in DMEM. Cells were studied for expression of *hMRS3/4*~EGFP 46–72 h post-transfection by confocal laser scanning microscopy (CLSM) as described previously [10]. Prior to analysis the cells were loaded for 20 min with 250 ng/ml MitoTracker Red CMXRos (Molecular Probes, Eugene, OR, USA) in DMEM+ at 37°C, washed with medium and incubated for further 20 min in DMEM+ at 37°C. Cells were monitored for EGFP- and MitoTracker-fluorescence using a Leica CLSM (Leica Lasertechnik, Heidelberg, Germany). The coverslips were placed in a perfusion chamber mounted on a Leica Fluovert FU inverted microscope (Leica Lasertechnik, Heidelberg, Germany), at 37°C. The following settings were used: 100×/1.3 oil Leitz Fluotar objective lens, excitation using a krypton-argon laser (bands 488 and 568 nm), excitation filter KP590, double-dichroic mirror 488/568, and emission filter HQ525/50 for EGFP and a long-pass 580-nm filter for MitoTracker Red, respectively. A beam-splitter at 580 nm was used to separate the EGFP and MitoTracker Red signals. Presentation images were generated using Adobe Photoshop version 4.0.

2.8. Transfection of *hMRS3/4* into yeast *mrs3^omrs4^o* and *mrs2^o* knock-out mutants

The *hMRS3/4* cDNA encoding the 364-aa protein and lacking only the non-conserved 5'-end (amplified with primers MRS4-41 and MRS4-21) was cloned into pCR2.1 (Invitrogen, San Diego, CA, USA). The insert was further amplified with *hMRS4-SacI* and *hMRS4-HindIII* (Table 1). The PCR product was digested with restriction enzymes *SacI* and *HindIII* and cloned into the corresponding sites of the vector pVT-U [11] creating plasmid pVT-U-hMRS3/4. The pVT-U-hMRS3/4 plasmid was digested with restriction enzyme *SphI* and the fragment containing *hMRS3/4* with *ADH1* gene promoter and *ADH1* 3' untranslated region was cloned into the *SphI* site of YC-plac22 and YEplac12 vectors [12] creating plasmids YCp-lac22-hMRS3/4 and YEplac12-hMRS3/4, respectively. Plasmid pVT-U-hMRS3/4 was transformed into yeast *S. cerevisiae* strain DBY747/*mrs2-1* Δ [13] and plasmids YCp-lac22-hMRS3/4 and YEplac12-hMRS3/4 were transformed into yeast *S. cerevisiae* strain DBY747/*mrs3^omrs4^o* (GW6/gd34) [2].

3. Results

3.1. Genomic structure and cDNA of *hMRS3/4* gene

The *hMRS3/4* cDNAs of 1448 bp and 1889 bp were con-

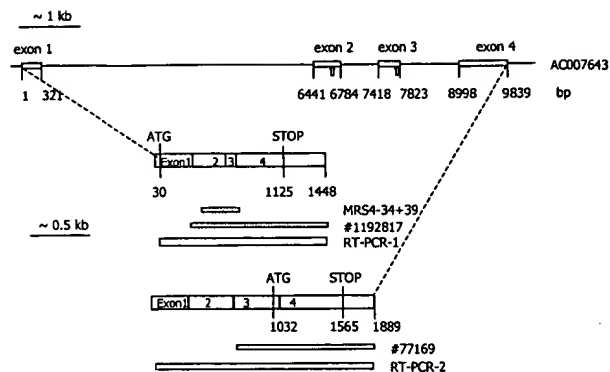


Fig. 1. Genomic organization and cDNA composition of *hMRS3/4*. The gene spans ~10 kb of genomic DNA and contains four exons transcribed as (i) a 1448-bp splice variant with an estimated coding region of 1095 bp, corresponding to a polypeptide of 364 aa and (ii) a 1889-bp splice variant having an ORF encoding a 177-aa polypeptide. EST, cDNA clone and RT-PCR sequences used in constructing the full-length cDNAs are shown below each splice variant. The cDNA sequence constitutions of the two different probes for Northern analysis are shaded. Arrows below exons 2 and 3 show alternative splice sites.

structed from overlapping EST sequences available in GenBank, cDNA clone sequences representing these ESTs, and sequencing of RT-PCR products. Comparing the cDNA sequences with the genomic sequence of the locus revealed that the gene consists of four exons spanning about 10 kb of genomic DNA (Fig. 1). Exons 2–4 corresponded to the EST and cDNA clone sequences and consequently also to the transcribed sequences predicted from the genomic sequence with program EbEST. Also the program Genscan at MIT succeeded in correctly predicting the internal and terminal exons of *hMRS3/4*. The first exon was predicted with program Genexpress from genomic sequence AC007643, and its presence was confirmed by RT-PCR. Additionally, three vertebrate ESTs homologous to yMRS3 and yMRS4 (GenBank accession BE012485, AW326482 and AA104365) were homologous to the predicted human exon 1. The first and the third intron splicing of *hMRS3/4* followed the conventional GT-AG rule, whereas the second did not. cDNA- and RT-PCR sequences revealed that the exons 2 and 3 are alternatively spliced with either (i) the 5'-end of exon 2 (228 bp) and 3'-end of exon 3 (58 bp) in the transcript (1448 bp variant) or (ii) full-length exons 2 and 3 in the transcript (1889-bp variant; Fig. 1). Both of these variants are supported by several ESTs from man, mouse, and cow, and by sequencing of RT-PCR products. The 1448-bp splice variant encodes a polypeptide of 364 aa in length. The likely initiator ATG in this cDNA occurs at nucleotide 30, is followed by an ORF of 1095 bp with a stop codon at nt 1125, and a polyA-signal at nt 1431. The 1889-bp splice variant possesses an ORF encoding a 177-aa polypeptide, identical to the C-terminus of the 364-aa protein (Figs. 1 and 2). Fig. 1 schematically shows the genomic structure of *hMRS3/4* and the composition of the cDNA splice variants. Exact nucleotide and amino acid sequences of the splice variants are available in public databases (GenBank accession numbers AF327402 and AF327403; EMBL accession numbers AJ303077 and AJ303078).

3.2. Mapping *hMRS3/4*

To accurately map *hMRS3/4*, the G3 radiation hybrid panel and a YAC contig on 10q24 were analyzed using *hMRS3/4*-specific PCR primers. A single product was amplified with MRS4-5 and -8 (Table 1) from total human DNA and from eight samples (no. 1, 44, 51, 57, 60, 64, 79, 82) of the radiation hybrid DNAs, while no PCR product was obtained in the other hybrids, mouse or hamster DNA. Combining Chromosome 10 RH Map, SHGC Chr.10 Radiation Hybrid Map (G3) and RH Consortium Gene Map '98-Chr. 10 (G3 panel; <http://www.gdb.org/>) data revealed that *hMRS3/4* maps close to the DNA marker D10S198, which matches well with the transcript map (<http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96>). This was confirmed by STS content mapping with *hMRS3/4*-specific primers of CEPH-mega-YAC contig representing the 10q24 region (data not shown).

3.3. Expression pattern of *hMRS3/4* in tissues

Northern hybridization analyses utilizing the insert of the IMAGE cDNA clone 1192817 as a probe identified a major *hMRS3/4* transcript of approximately 1.8 kb in all tissues examined (placenta, lung, kidney, pancreas, liver, brain, skeletal muscle and heart), with the strongest signals from heart, skeletal muscle, and liver (Fig. 2). A similar expression pattern was seen in the hybridization with MRS4-39+34 RT-PCR product, which was specific to the cDNA of the 364-aa form (results not shown). In all hybridizations, weak signals of approximately 3.2 and 4.4 kb were observed in all tissues, except that the 4.4-kb signal was absent in the brain. These signals most probably result from non-specific hybridization or transcripts with a high sequence homology with *hMRS3/4*. The 1.8-kb signal corresponds to the length of the cDNA splice variant encoding the 177-aa partial protein. No signal corresponding to the 1448-bp cDNA encoding the 364-aa variant could be detected. However, several other lines of evidence clearly show that the 1448-bp cDNA encodes the functional protein variant. The absence of Northern signal may indicate that we are still lacking some of the 5' untranslated sequence of the 364-aa cDNA, and that the two cDNA signals overlap with each other in Northern (Fig. 2).

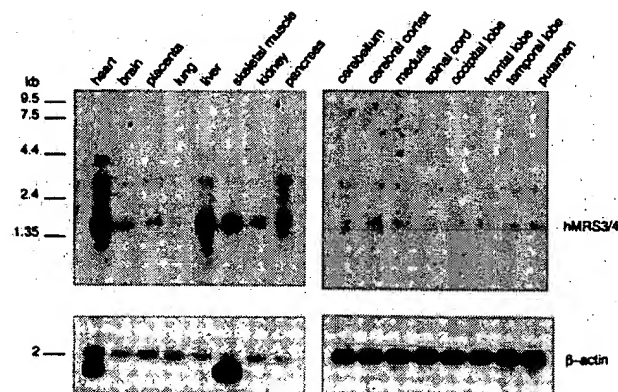


Fig. 2. Northern blot analysis of *hMRS3/4*. Hybridization with the insert of IMAGE cDNA clone 1192817 as a probe revealed highest expression levels in heart, skeletal muscle, and liver (left: MTN-blot; middle: brain-MTN-blot). The equal loading of RNA on lanes was confirmed by probing the same membrane with β -actin.

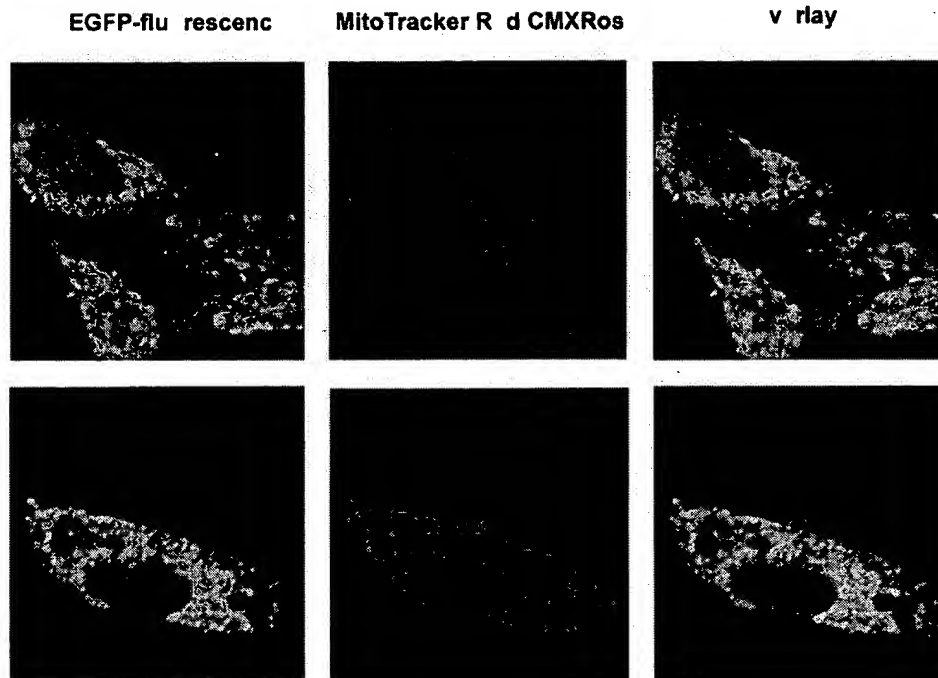


Fig. 3. Mitochondrial localization of the 177-aa (upper pictures) and 364-aa (lower pictures) hMRS3/4 protein variants. HEK293 cells, transfected with pRSV.hMRS3/4~EGFP, were loaded with MitoTracker Red CMXRos. Fluorescence images were obtained by CLSM as described in Section 2. The green color is used as the digital pseudocolor for the fluorescence emitted by hMRS3/4-177 or -364~EGFP, red is the digital pseudocolor for fluorescence emitted by MitoTracker Red, and yellow results from their overlap, indicating the co-localization of hMRS3/4~EGFP and MitoTracker Red in mitochondria of transfected HEK293 cells.

3.4. Mitochondrial targeting of hMRS3/4

To test the subcellular localization of hMRS3/4, its coding sequence for the 364-aa and the 177-aa protein were tagged with EGFP. MitoTracker Red CMXRos was used as a mitochondrial marker. The signals of MitoTracker and hMRS3/4-177 and -364~EGFP co-localized in the mitochondria of transfected HEK293 cells, showing that both proteins are targeted into the mitochondria (Fig. 3).

3.5. hMRS3/4 structure and conservation

BLAST searches with the 364-aa hMRS3/4 as a query sequence against SwissProt database showed 38% identity and 55% similarity with the yMRS4 ($E = 7e-53$), and 37% identity and 52% similarity with the yMRS3 ($E = 1e-52$). Programs ProDom, PredictProtein, ProfileScan, and Tmpred suggested that the 364-aa protein contains six transmembrane helical domains conserved in MCF proteins between species (Fig.

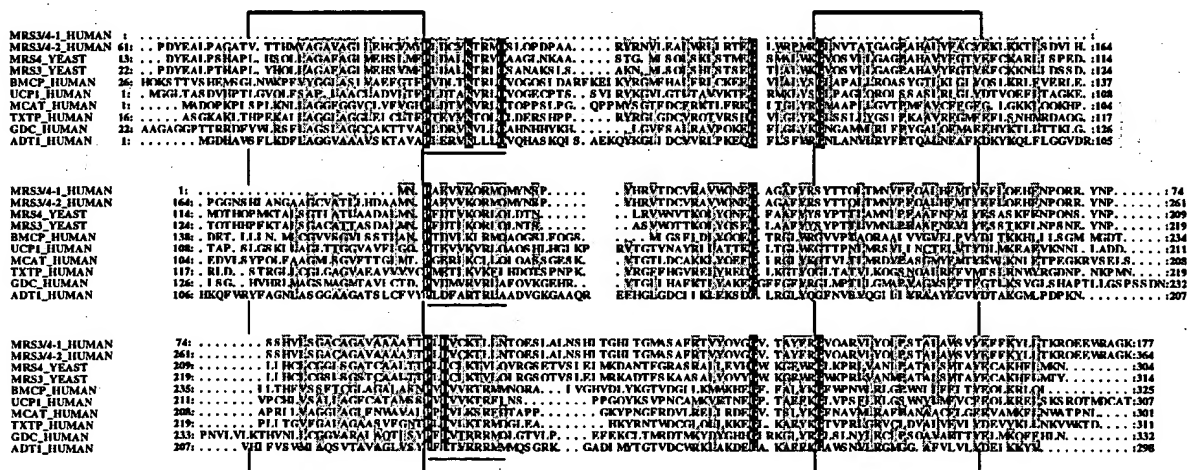


Fig. 4. Multiple-alignment of hMRS3/4 with other members of MCF. The multiple-alignment was performed with ClustalX and improved with MacBoxshade. The boxes indicate the conservative transmembrane domains [2]. The sites of the conservative signature sequence are underlined. MRS: mitochondrial RNA splicing; MRS3/4-1: 177-aa variant; MRS3/4-2: 364-aa variant; BMCP: brain mitochondrial carrier protein; UCP: brown fat uncoupling protein; MCAT: mitochondrial carnitine/acylcarnitine translocase; TXTP: tricarboxylate transport protein; GDC: Grave's disease carrier protein; ADT: ATP/ADP translocase. The first 60, 12, 21, 25, 15, and 21 aa of hMRS3/4, yMRS4, yMRS3, human BMCP, human TXTP and human GDC, respectively, are deleted for convenience of the alignment.

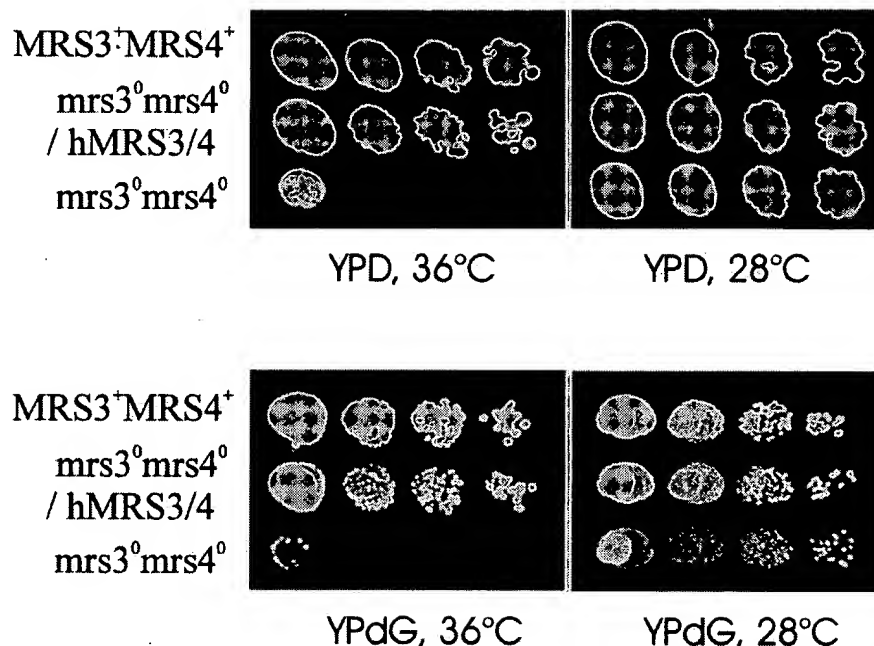


Fig. 5. Functional complementation of the yeast *mrs3°mrs4°* strain. Yeast *S. cerevisiae* strain DBY747*mrs3°mrs4°* was transformed with vector YEplac112-*hMRS3/4* containing *hMRS3/4* driven by the yeast constitutive *ADHI* promoter. The transformants (*mrs3°mrs4°/hMRS3/4*), untransformed strain (*mrs3°mrs4°*) and isogenic wild type strain DBY747 (*MRS3+MRS4+*) were suspended in water and 10-fold dilution series were spotted on plates containing glycerol as non-fermentable carbon source supplemented with 0.1% glucose (YPdG) or on glucose medium (YPD). Plates were incubated at 28 or 36°C for 5 days. *hMRS3/4* could restore the growth defect of yeast *mrs3°mrs4°* mutants.

4; Pfam accession PF00153). Typical of a MCF protein with internal targeting signals, no N-terminal targeting signal was recognized in the polypeptide. Three mitochondrial energy transfer protein signatures were identified in the protein (Prosite accession number GC0435, PDOC00189, and PS00215) [2,3], and the protein also contained two prokaryotic membrane lipoprotein attachment sites (Prosite accession number PS00013). The N- and C-termini of the 364-aa protein were predicted to face the mitochondrial intermembrane space. The 177-aa splice variant of *hMRS3/4* was predicted to contain three complete transmembrane domains, identical to the C-terminus of the 364-aa protein.

3.6. Transfection of *hMRS3/4* into a yeast *mrs3°mrs4°* and *mrs2°* knock-out mutants

The double knock-out mutations of yeast genes *MRS3* and *MRS4* (*mrs3°mrs4°*) caused a temperature-sensitive growth phenotype in yeast cells, i.e. growth inhibition at 36°C both on fermentable (YPD) and non-fermentable (YPdG) substrates, whereas growth at 28°C was only slightly affected (Fig. 5). Single knock-out mutations (*mrs3°*, *mrs4°*) did not show any obvious growth defect (not shown). When transfected with multi-copy plasmid YEplac112-*hMRS3/4*, expressing *hMRS3/4* in yeast under a constitutive promoter, *mrs3°mrs4°* double knock-out mutant regained growth at 36°C both on fermentable and non-fermentable substrates, indicating that *hMRS3/4* can substitute its yeast counterparts. Expression of the same *hMRS3/4* construct from a low-copy, centromeric plasmid (YCplac22-*hMRS3/4*) also restored growth of the *mrs3°mrs4°* mutant, although to a lesser degree (not shown). Overexpression of *yMRS3* and *yMRS4* have been reported to suppress defects in RNA splicing and cytochrome biogenesis of yeast with *mrs2-1* mutation, a gene in-

involved in mitochondrial Mg^{2+} homeostasis [13,14]. When *hMRS3/4* was expressed in yeast from a multi-copy vector, it also suppressed the growth defect of the *mrs2-1* knock-out mutant, although less efficiently than *yMRS3* and *yMRS4* (data not shown).

3.7. Analysis of patient samples

To study the possible involvement of *hMRS3/4* in the pathogenesis of either adPEO or IOSCA, the exons, including the splice sites, were PCR-amplified and sequenced from the genomic DNA samples of two adPEO patients, one IOSCA patient and two control subjects. No sequence variations between the patients and controls could be identified.

4. Discussion

We characterize here a novel human mitochondrial protein, *hMRS3/4*. It is structurally and functionally homologous to the yeast mitochondrial proteins *MRS3* and *MRS4*, two members of the mitochondrial carrier protein family, suggested to translocate solutes across the inner mitochondrial membrane [2]. MCF proteins are characterized by three repeats of about 100 aa each, with two highly conserved transmembrane helices per repeat. The human homolog of *yMRS3* and *yMRS4* consists of 364 aa, contains six putative transmembrane domains, and shares structural features with mitochondrial inner membrane solute carriers. The structure is typical of MCF, harboring no cleavable preproteins for mitochondrial targeting, but possessing internal targeting information [15,16].

Single and double knock-out mutations of *yMRS3* and *yMRS4* have been previously reported not to affect growth of yeast cells at 28°C on fermentable or non-fermentable carbon sources [2]. We show here, however, that the *mrs3°mrs4°*

double mutation in yeast causes temperature-sensitive growth inhibition: growth at 28°C was only slightly affected, but at 36°C it was strongly reduced on both substrates. Since single knock-out mutations did not show a growth defect on either temperature, the presence of either yMRS3 or yMRS4 is sufficient to sustain normal growth of yeast cells. The observed phenotype of the double knock-out mutant is remarkable, since most knock-out mutations in nuclear-encoded mitochondrial genes in yeast cause a growth defect on non-fermentable substrates only. The few genes known to be essential for growth on fermentable substrates are all involved in mitochondrial protein or metabolite transport [17]. The fact that the overexpression of yMRS3, yMRS4 and hMRS3/4 can restore growth and mitochondrial metal ion concentrations also in yeast lacking the putative yeast mitochondrial Mg^{2+} transporter MRS2 ([13,14]; Gregan and Schweyen, manuscript in preparation) suggests a role in mitochondrial metal ion transport or homeostasis. The human homolog hMRS3/4 expressed in yeast *mrs3⁰mrs4⁰* mutant cells can substitute for its yeast homologs and restore cell growth, which is consistent with the conservation of the transport function from yeast to man, and in line with observations on some other mitochondrial carriers [18].

hMRS3/4 is ubiquitously expressed showing the highest expression in the heart, skeletal muscle and liver. The lack of the 1.4-kb transcript on Northern hybridization analyses suggests that we may still miss a part of the 5' untranslated region of the gene. However, homology analyses strongly support the start codon in the 1.4-kb cDNA to be the one suggested, and the yeast complementation assay shows that the expressed protein is functional. The existence of the 1.9-kb splice variant encoding the 177-aa polypeptide is supported by numerous EST sequences in GenBank, and the cDNA is readily amplified by RT-PCR in RNA extracts. The polypeptide is interrupted in the middle of a conserved transmembrane region, when compared with the structures of yMRS3 and yMRS4, lacking 2.5 transmembrane domains of the 364-aa protein. In spite of this, the 177-aa form is effectively targeted into the mitochondria, in which it could possibly serve a transporter role as a dimer. The high homology of both variants to the yeast MRS3 and 4, previously shown to be integral inner membrane proteins of mitochondria [2], together with the targeting of both products into the mitochondria, strongly suggest that hMRS3/4 is a mitochondrial protein.

hMRS3/4 is located on chromosome 10q24, which also harbors disease gene loci for adPEO [4,5] and IOSCA [6,7]. The tissues of adPEO patients accumulate multiple mtDNA deletions, although the primary gene defect is nuclear [4]. A recent report of mutations in the heart- and muscle-specific isoform of adenine nucleotide translocator underlying the chromosome 4-linked adPEO [19] made *hMRS3/4* a good candidate gene for the chromosome 10-linked disease form, since both the proteins belong to the MCF. In addition, the mitochondrial expression pattern of *hMRS3/4*, i.e. ubiquitous expression with increased expression levels in skeletal muscle and heart, suggested a putative role in a mitochondrial disease. The adPEO locus overlaps with that of IOSCA [6,7], an autosomal recessively inherited disease resembling closely another recessive mitochondrial disorder, Friedreich's ataxia (reviewed in [20]), and sharing a spectrum of symptoms with

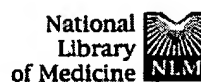
PEO. We did not detect mutations in *hMRS3/4* in any of our patients, rendering it unlikely as the disease gene underlying these disorders.

The high homology of hMRS3/4 with other MCF members, as well as the common signature sequences, its mitochondrial location and the ability to replace the functions of the homologous yeast proteins, strongly support that it belongs to the MCF and shares the role of its yeast counterparts in human cells i.e. the transport of ions across the inner mitochondrial membrane. Its essential role for growth in yeast cells and conservation across species indicate that the MRS proteins are important for eukaryotes. Further studies on the function of hMRS3/4 may provide new insights in the functions of these MCF proteins in general.

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☐ 1: Ann Neurol. 2003 Jun;53(6):711-7.

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Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death.

Sullivan PG, Dube C, Dorenbos K, Steward O, Baram TZ.

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Department of Neurobiology and Behavior, University of California at Irvine, Irvine, CA 92697-4475, USA.

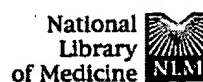
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Excitotoxic cell death is the fundamental process responsible for many human neurodegenerative disorders, yet the basic mechanisms involved are not fully understood. Here, we exploited the fact that the immature brain is remarkably resistant to seizure-induced excitotoxic cell death and examined the underlying protective mechanisms. We found that, unlike in the adult, seizures do not increase the formation of reactive oxygen species or result in mitochondrial dysfunction in neonatal brain, because of high levels of the mitochondrial uncoupling protein (UCP2). UCP2 expression and function were basally increased in neonatal brain by the fat-rich diet of maternal milk, and substituting a low-fat diet reduced UCP2, restored mitochondrial coupling, and permitted seizure-induced neuronal injury. Thus, modulation of UCP2 expression and function by dietary fat protects neonatal neurons from excitotoxicity by preventing mitochondrial dysfunction. This mechanism offers novel neuroprotective strategies for individuals, greater than 1% of the world's population, who are affected by seizures.

PMID: 12783416 [PubMed - indexed for MEDLINE]

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☐ 1: J Mol Med. 2003 May;81(5):327-32. Epub 2003 Mar 28. Related Articles, Links

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Uncoupling protein 1 and 3 polymorphisms are associated with waist-to-hip ratio.

Herrmann SM, Wang JG, Staessen JA, Kertmen E, Schmidt-Petersen K, Zidek W, Paul M, Brand E.

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Department of Clinical Pharmacology, Benjamin Franklin Medical Center, Freie Universitat Berlin, Hindenburgdamm 30, 12200 Berlin, Germany.

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Body weight regulation is a complex phenotype also depending on the action of uncoupling proteins (UCPs) that mediate the "uncoupling" of respiration leading to the dissipation of energy as heat. This study investigated whether genetic variants in the genes encoding UCP-1 and UCP-3 are associated with different obesity-related phenotypes in 162 whites with a wide range of body mass index. All subjects were genotyped for the polymorphisms UCP-1 A-3826G, UCP-1 Ala64Thr, and UCP-3 C-55T using a PCR-based restriction method with appropriate enzymes. The frequencies of the UCP-1 3826G, UCP-1 64Thr, and UCP-3 55T alleles were 27.2%, 12.0%, and 22.8%, respectively. No significant associations were observed between polymorphism and body mass index or obesity. However, after adjustment for gender, age, body mass index, and diabetes mellitus the waist-to-hip ratio was significantly associated with UCP-1 Ala64Thr ($P=0.003$) and UCP-3 C-55T ($P=0.02$) but not with UCP-1 A-3826G. The higher waist-to-hip ratios associated with the UCP-1 64Thr and UCP-3 55T alleles were due to higher waist circumference in these allele carriers. In conclusion, central obesity in whites as reflected by an increased waist-to-hip ratio is associated with the UCP-1 Ala64Thr and UCP-3 C-55T polymorphisms. To what extent these genotypes contribute to the overall cardiovascular risk remains to be elucidated.

PMID: 12756473 [PubMed - in process]

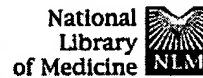
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PubMed☐ 1: Obes Rev. 2000 Oct;1(2):61-72.[Related Articles, Links](#)

The human uncoupling protein-1 gene (UCP1): present status and perspectives in obesity research.

Del Mar Gonzalez-Barroso M, Ricquier D, Cassard-Doulcier AM.

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Centre de Recherches sur l'Endocrinologie Moléculaire et le Développement,
CNRS, 9 rue Jules Hetzel, 92190 Meudon, France.

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Energy expenditure through brown adipose tissue thermogenesis contributes either to maintenance of body temperature in a cold environment or to wasted food energy, i.e. cold-induced or diet-induced thermogenesis. Both mechanisms are due to a specific and unique protein: the uncoupling protein-1. Uncoupling protein-1 is exclusively expressed in mitochondria of brown adipocytes where it uncouples respiration from ATP synthesis, dissipating the proton gradient as heat. In humans, although uncoupling protein-1 can be detected, the inability to quantify brown adipose tissue makes it difficult to argue for a role for uncoupling protein-1 in thermogenesis and energy expenditure. This review summarizes data supporting the existence of brown adipocytes and the role of UCP1 in energy dissipation in adult humans. Understanding the mechanisms which regulate transcription and expression of the human UCP1 gene will facilitate the identification of molecules able to increase the levels of this protein in order to modulate energy expenditure in adult humans.

Publication Types:

- Review
- Review, Tutorial

PMID: 12119988 [PubMed - indexed for MEDLINE]

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A marked upregulation of uncoupling protein 2 gene expression in adipose tissue of hyperthyroid subjects.

Hoffstedt J, Folkesson R, Wahrenberg H, Wennlund A, van Harmelen V, Arner P.

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Department of Medicine, Karolinska Institutet, Huddinge University Hospital, Sweden. johan.hoffstedt@medhs.ki.se

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Recently, a family of uncoupling protein (UCP) genes has been discovered. The role of these genes is unknown, but it has been suggested that they are involved in regulating resting metabolic rate. In this study, we hypothesised that thyroid hormone status may influence the expression of UCP2 mRNA. The adipose tissue levels of UCP2 mRNA were measured in eight female subjects before and after treatment for thyrotoxicosis. All subjects in the hyperthyroid condition had markedly enhanced plasma levels of thyroxine (62.0 ± 6.9 vs. 17.9 ± 1.7 , $p = 0.012$) and triiodothyronine (37.9 ± 6.9 vs. 5.9 ± 0.9 , $p = 0.012$), accelerated heart rate (94 ± 7 vs. 69 ± 5 , $p = 0.012$), decreased BMI (24.5 ± 1.9 vs. 25.1 ± 1.9 , $p = 0.025$) and decreased percentage body fat (32.8 ± 4.4 vs. 37.1 ± 4.5 , $p = 0.018$), as compared to the euthyroid state. Using RT-competitive-PCR, the UCP2 mRNA levels were found to be 2.5-fold upregulated in hyperthyroidism (10.4 ± 1.7 vs. 4.2 ± 1.3 amol/microg RNA, $p = 0.012$). In contrast, no difference in expression levels of the reference gene 18SrRNA was seen in the hyperthyroid versus the euthyroid state (317 ± 49 vs. 279 ± 25 amol/microg RNA, $p = 0.48$) but the difference in UCP2 mRNA levels between the hyper- and euthyroid state remained when UCP2 was related to 18SrRNA ($p = 0.012$). In conclusion, thyrotoxicosis markedly increases the expression of UCP2 mRNA in adipose tissue, which suggests a role for thyroid hormones in the regulation of this uncoupling protein in man.

PMID: 11246812 [PubMed - indexed for MEDLINE]

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☐ 1: Diabetologia. 2000 Dec;43(12):1558-64.

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Evidence that single nucleotide polymorphism in the uncoupling protein 3 (UCP3) gene influences fat distribution in women of European and Asian origin.

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Cassell PG, Saker PJ, Huxtable SJ, Kousta E, Jackson AE, Hattersley AT, Frayling TM, Walker M, Kopelman PG, Ramachandran A, Snehalatha C, Hitman GA, McCarthy MI.

Department of Diabetes and Metabolic Medicine, St Bartholomews and Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, London, UK.

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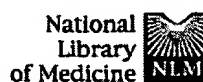
AIMS/HYPOTHESIS: Uncoupling proteins are mitochondrial transmembrane carriers implicated in the regulation of energy balance. Dysfunction of UCP3 (the predominant uncoupling protein in skeletal muscle) might therefore be expected to reduce thermogenic capacity, alter energy homeostasis and influence predisposition to obesity and Type II (non-insulin-dependent) diabetes mellitus. A variant in the putative promoter region of UCP3 (-55 c-->t) has recently been identified, and an association with obesity reported in French subjects. Our aim was to study the pathophysiological role of this variant in diabetes-related and obesity-related traits using two distinct ethnic populations. **METHODS:** The -55 c-->t variant was genotyped in 85 South Indian and 150 European parent-offspring trios ascertained through Type II diabetic probands and in 455 South Indian subjects initially recruited to an urban survey into the prevalence of diabetes. **RESULTS:** In South Indian and European parent-offspring trios there was no preferential transmission of either allele at the -55 c-->t polymorphism to diabetic offspring (South Indians, $p = 0.60$; Europeans, $p = 0.15$). When family members were analysed for intermediate traits, the t-allele was associated with increased waist-to-hip ratio but only in females (South Indian mothers $p = 0.036$, daughters $p = 0.032$; European mothers $p = 0.037$, daughters $p = 0.14$). These findings were replicated in South Indian females from the population-based survey ($p = 0.039$). **CONCLUSION/INTERPRETATION:** The consistent association between the t-allele at this locus and increased waist-to-hip ratio in women from three separate data sets indicates that variation at this polymorphism (or another locus with which it is in linkage disequilibrium) influences fat distribution but that this effect is restricted to females.

PMID: 11151767 [PubMed - indexed for MEDLINE]

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Regulation of uncoupling protein (UCP) 2 and 3 in adipose and muscle tissue by fasting and growth hormone treatment in obese humans.

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Pedersen SB, Borglum JD, Kristensen K, Norrelund H, Otto J, Jorgensen L, Richelsen B.

Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus Amtssygehus, DK-8000 Aarhus C, Denmark. amtssp@aaau.dk

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OBJECTIVE: To investigate whether the expression of uncoupling proteins (UCP2 and UCP3) was affected by a very low calorie diet (VLCD) and growth hormone (GH) treatment for 4 weeks. **DESIGN:** A randomized, placebo-controlled intervention study of VLCD with or without concomitant GH-treatment. **SUBJECTS:** Seventeen obese women (body mass index, BMI=42.1+/-1.4 kg/m² (range 31.8-54.5 kg/m²)) treated with VLCD for 4 weeks and randomized to concomitant placebo treatment (n=9) or GH treatment (n=8). **MEASUREMENTS:** Fat mass and lean body mass were measured by dual-energy X-ray absorptiometry. Energy expenditure (EE) was measured by indirect calorimetry. UCP2 and UCP3 mRNA were measured in adipose tissue and skeletal muscle biopsies before VLCD and after VLCD+/-GH-treatment by reverse transcription polymerase chain reaction (RT-PCR). **RESULTS:** VLCD treatment resulted in a mean weight loss of 5.23 kg+/-0.8 (P<0.01), a 4.1% decrease in EE (P<0.05) and a 24% decrease in UCP3 mRNA in adipose tissue (P<0.03), whereas adipose tissue UCP2 mRNA and skeletal muscle UCP2 and UCP3 mRNA levels were unchanged. GH-treatment had no effects on EE, changes in body weight or UCP mRNA level. In multiple regression analysis the change in EE caused by VLCD was significantly correlated with changes in adipose tissue UCP2 mRNA (r=0.66, P<0.02) and a tendency towards a significant association with the change in adipose tissue UCP3 mRNA (r=0.45, P=0.09), but not with change in body weight, skeletal muscle UCP2 or UCP3 mRNA levels. **CONCLUSION:** VLCD for 4 weeks decreased UCP3 mRNA expression in human adipose tissue, whereas GH-treatment had no effect on UCP expression. Multiple regression analysis demonstrated that changes in adipose tissue UCP2 and probably UCP3 mRNA were correlated with the change in EE. These findings indicate that UCPs in adipose tissue in very obese individuals might play a role for the reduction in EE observed during energy restriction.

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

PMID: 10951534 [PubMed - indexed for MEDLINE]

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Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma.

Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warden CH, Castilho RF, Melcher T, Gonzalez-Zulueta M, Nikolich K, Wieloch T.

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[1] Wallenberg Neuroscience Center, BMC A13, 221 84 Lund, Sweden. [2] These authors contributed equally to this work.

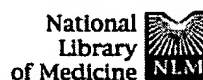
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Whereas uncoupling protein 1 (UCP-1) is clearly involved in thermogenesis, the role of UCP-2 is less clear. Using hybridization, cloning techniques and cDNA array analysis to identify inducible neuroprotective genes, we found that neuronal survival correlates with increased expression of Ucp2. In mice overexpressing human UCP-2, brain damage was diminished after experimental stroke and traumatic brain injury, and neurological recovery was enhanced. In cultured cortical neurons, UCP-2 reduced cell death and inhibited caspase-3 activation induced by oxygen and glucose deprivation. Mild mitochondrial uncoupling by 2,4-dinitrophenol (DNP) reduced neuronal death, and UCP-2 activity was enhanced by palmitic acid in isolated mitochondria. Also in isolated mitochondria, UCP-2 shifted the release of reactive oxygen species from the mitochondrial matrix to the extramitochondrial space. We propose that UCP-2 is an inducible protein that is neuroprotective by activating cellular redox signaling or by inducing mild mitochondrial uncoupling that prevents the release of apoptogenic proteins.

PMID: 12858170 [PubMed - as supplied by publisher]

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Uncoupling Protein-2 Overexpression Inhibits Mitochondrial Death Pathway in Cardiomyocytes.

Teshima Y, Akao M, Jones SP, Marban E.PubMed
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Institute of Molecular Cardiobiology, The Johns Hopkins University, Baltimore, Md.

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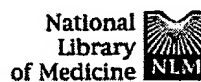
Uncoupling proteins (UCPs) are located in the mitochondrial inner membrane and partially dissipate the transmembrane proton electrochemical gradient. UCP2 is expressed in various human and rodent tissues, including the heart, where its functional role is unknown. In the present study, we tested the hypothesis that UCP2 overexpression could protect cardiomyocytes from oxidative stress-induced cell death by reducing reactive oxygen species (ROS) production in mitochondria. Using an adenoviral vector containing human UCP2, we investigated the effects of UCP2 overexpression on the mitochondrial death pathway induced by oxidative stress (100 micro mol/L H₂O₂) in cultured neonatal cardiomyocytes. UCP2 overexpression significantly suppressed markers of cell death, including TUNEL positivity, phosphatidylserine exposure, propidium iodide uptake, and caspase-3 cleavage. Furthermore, UCP2 remarkably prevented the catastrophic loss of mitochondrial inner membrane potential induced by H₂O₂, which is a critical early event in cell death. Ca(2+) overload and the production of ROS in mitochondria, both of which contribute to mitochondrial inner membrane potential loss, were dramatically attenuated by UCP2 overexpression. Thus, overexpression of UCP2 attenuates ROS generation and prevents mitochondrial Ca(2+) overload, revealing a novel mechanism of cardioprotection.

PMID: 12855674 [PubMed - as supplied by publisher]

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- [Am J Clin Nutr. 2002 Apr;75\(4\):714-9.](#)

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Uncoupling protein genes and racial differences in obesity.

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Polednak AP.

Publication Types:

- Comment
- Letter

PMID: 12791636 [PubMed - indexed for MEDLINE]

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Mitochondrial uncoupling protein 2 in the central nervous system: neuromodulator and neuroprotector.

Horvath TL, Diano S, Barnstable C.

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Department of Obstetrics and Gynecology, Yale University School of Medicine,
333 Cedar Street, FMB 339, New Haven, CT 06520, USA.
tamas.horvath@yale.edu

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Uncoupling proteins (UCPs) are localized in the inner membrane of the mitochondria in diverse tissues and decrease mitochondrial membrane potential. The first of these proteins, UCP1, was discovered in brown adipose tissue, where it has a well-described role in thermogenesis. The functional significance of other UCPs, including UCP2, is less well understood. Here we summarize the recent advancements on the role of UCP2 in the brain and portray this uncoupler as an important player in normal neuronal function as well as a key cell death-suppressing device. These previously unknown functions of UCPs offer new avenues not only for the better understanding of these proteins but also for the furthering of our knowledge on the central nervous system in healthy and disease states.

Publication Types:

- Review
- Review, Tutorial

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